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The ommatidia of *Arca noae*: a three-tier structure with a central light-guiding element for the receptor cell

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Abstract The compound eyes of ark clams appear to function as an optical system to trigger shell closure against predators. We have analyzed the structure of the ommatidia of *Arca noae* by thin section electron microscopy and serial sectioning, Concanavalin A–gold labeling and acid phosphatase cytochemistry. Our results demonstrate that the ommatidia are a three-tier structure composed of a central single receptor cell, surrounded and covered by proximal pigment cells followed by rows of distal pigment cells. The receptor cells of *Arca noae* have no lens and the disks of their receptive segment are derived from sensory cilia. The distal mitochondrial segment in the cytoplasm between the nucleus and the receptive segment is surrounded by a mass of Concanavalin A-reactive glycogen particles. Although both, proximal and distal pigment cells have numerous microvilli, only those of the proximal pigment cells form a well-aligned brush border. The microvilli of the latter are ≈9–11 μm long and have a diameter of ≈70–80 nm. Numerous microlamellar bodies cover them. The microlamellar bodies are stored in acid phosphatase-negative secretory granules of the pigment granule-free apical cytoplasm of proximal pigment cells before their secretion. Observation of living compound eyes indicated that the apex of proximal pigment cells transmitted significantly more light than the surrounding distal pigment cells. Hence, the regular geometry of the brush border seems to be a light-guiding structure for receptor cells similar to an optical fiber.

Keywords *Arca noae* · Ommatidium · Pigment cell · Receptor cell · Brush border · Electron microscopy

Introduction

Recent evo-devo experiments indicate that all bilateria use the same master control gene, Pax 6, and the same subordinate Retinal Determination Gene Network (RDGN) for eye morphogenesis. Since a transcription factor like Pax 6 can regulate any target gene as long as it contains the appropriate *cis*-regulatory elements, the reason for this conservation is due to its evolutionary history. All the various eye-types found in ecdysozoa, lophotrochozoa, and chordata must go back to their last common ancestor, which used Pax 6 and the RDGN for eye morphogenesis. This indicates that all the various eye-types found in animals are despite of their structural and functional variation of monophyletic origin (reviewed in Gehring 2005; Gehring and Ikeo 1999). These findings lend support to Darwin's hypothesis that the various eye-types are derived from a simple prototype, consisting of a photoreceptor cell

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("nerve") and a pigment cell shielding the light from one side and therefore, allows directional vision. Such a prototypic eye was indeed found, for example in planarians and in trochophora larvae. Highly evolved eyes are found sporadically also in cnidaria, which do not have a Pax 6 gene, but they do have at least two other Pax genes, Pax B in scyphozoa and Pax A in hydrozoa, which are capable of inducing eyes and may be ancestors of Pax 6 (Kozmik et al. 2003; Suga et al. 2010). These medusae, which form lens eyes with ciliary photoreceptors, also express all the genes of the RDGN (Graziussi et al. 2011). In line with the phylogenetic evidence for the evolution of eyes from a common ancestor before the Cambrian "explosion", highly evolved compound eyes have been found in Cambrian trilobites and most recently in early Cambrian arthropods (Lee et al. 2011).

Compound eyes have been found not only in arthropods (ecdysozoa) but also in some mollusks (lophotrochozoa). In this study, we have carried out a detailed ultrastructural analysis of the compound eye of the ark clam (*Arca noae*), which has a large number of compound eyes along the edge of the mantel. These eyes may be considered as relatively primitive, since they lack lenses and the ommatidia consist of three cells only, one photoreceptor and two pigment cells. A comparative analysis of ark clams and fan worms has been carried out previously by Nilsson (1994) on Central American species. In this study, we concentrate on the Mediterranean ark clam (*Arca noae*).

Materials and methods

Ark clams

Ark clams were collected at the Laboratoire Arago in Banyuls-sur-Mer (France) and kept in seawater tanks at 18°C at the Biozentrum, University of Basel (Switzerland).

Live tissue observation

Carefully dissected edges of the mantel of Ark clams were directly observed and photographed under visible light epi-illumination.

Tissue fixation and resin embedding

Ark clams were opened with a spatula and the edge of the mantel containing the row-like aligned eyes was carefully dissected and immediately fixed. The tissue was fixed by immersion in 4% formaldehyde (freshly prepared from paraformaldehyde, Sigma-Aldrich, Buchs, Switzerland)—0.2% glutaraldehyde (vacuum-distilled, Sigma-Aldrich) in Hepes (40 mM, pH 8.0)-buffered seawater for 1 h at ambient temperature. After three rinses with PBS (10 mM phosphate buffer, pH 7.2, 0.15 M NaCl), small pieces of the mantel edge with a single compound eye were dissected under a microscope and postfixation in 1% phosphate-buffered (pH 7.2) OsO_4 or in 1% reduced OsO_4 (Karnovsky 1971) was performed for 30 min to 1 h at ambient temperature. Following rinses with PBS, tissue pieces were dehydrated in a series of ethanol and embedded in Epon-Araldite according to standard protocol.

Semithin (0.1–0.2 µm) and ultrathin (about 80 nm) sections were prepared with diamond knifes (Diatome, Biel, Switzerland) using an Ultracut S ultramicrotome (Leica Microsystems). Series of consecutive ultrathin sections were placed on slot grids. Ultrathin sections were stained with uranyl acetate and lead citrate and observed in a Zeiss 912 AB electron microscope (Carl Zeiss SMT AG) at 80 kV and photographed using 70 mm sheet film. Film sheets were subsequently digitized and images prepared using Adobe Photoshop CS3 software (Adobe Systems, Inc.).

Semithin sections were mounted on Superfrost® glass slides, stained with toluidine blue or methylene blue azur and examined using a Zeiss Axiophot photomicroscope equipped with a Zeiss AxioCam digital camera.

Acid phosphatase histochemistry

Tissue was fixed in 4% formaldehyde in Hepes-buffered seawater for 1 h at ambient temperature, rinsed with PBS and stored in PBS at 4°C until use. Acid phosphatase activity was demonstrated with cytidine 5′-monophosphate (Sigma, St. Louis, MO) as substrate according to the protocol of Robinson and Karnovsky (1983), which employs cerium chloride as capture reagent. Afterwards, tissue was

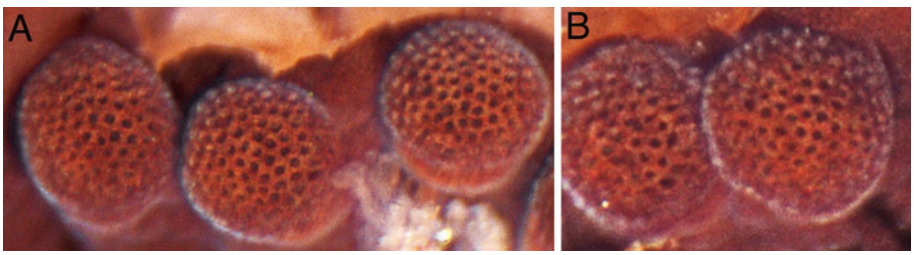

Fig. 1 By light microscopy, living compound eyes of *Arca noae* have a sponge-like appearance (**a**, **b**). The tunnel-like structures of the ommatidia in the periphery of the eyes refract incident light as indicated by the silver reflections

postfixed in 1% phosphate-buffered (pH 7.2) OsO_4 for 30 min at ambient temperature. Following rinses with PBS, tissue pieces were dehydrated in a series of ethanol and embedded in Epon-Araldite according to standard protocol. Thin sections were counterstained with uranyl acetate and lead citrate.

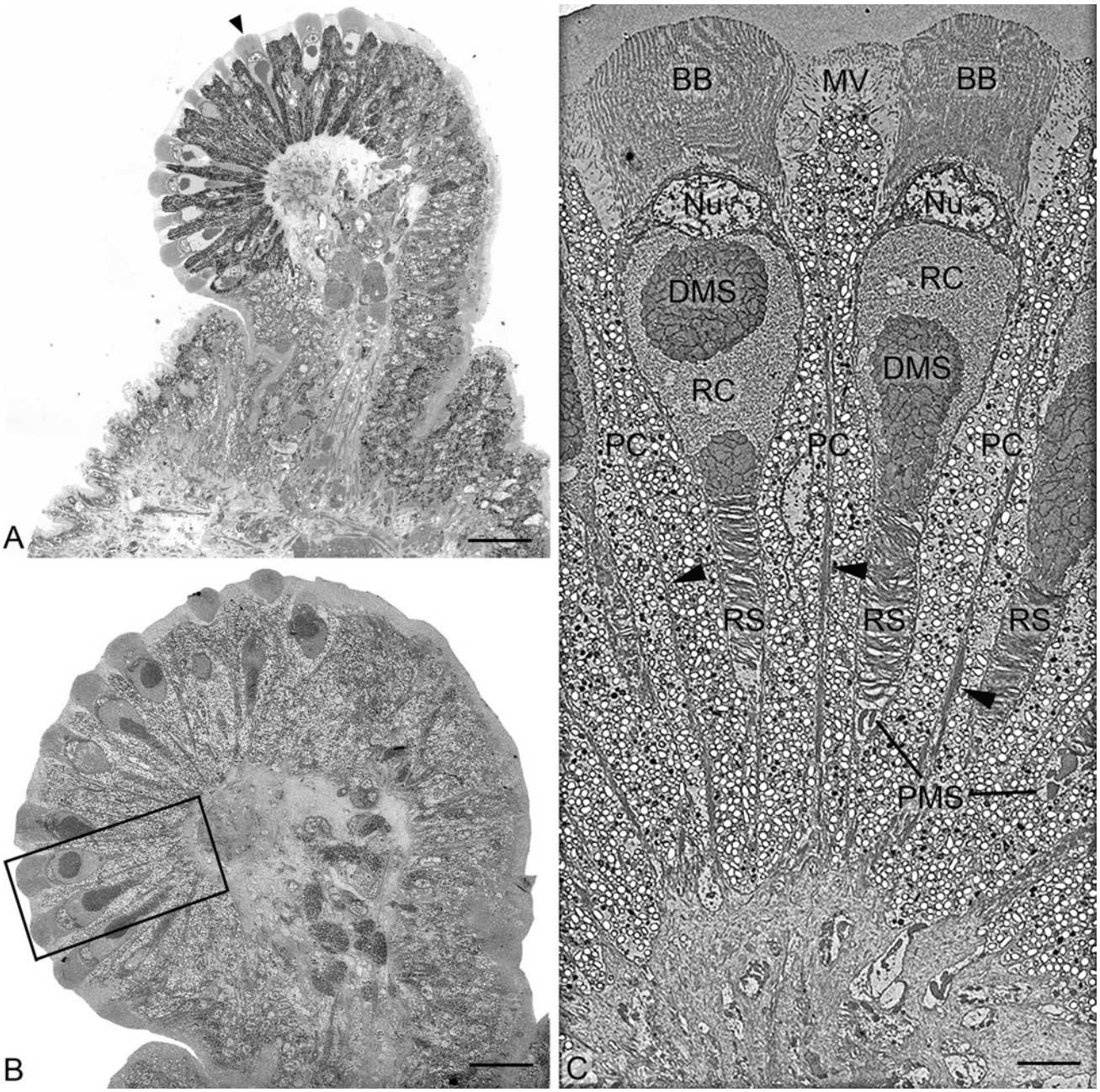

Fig. 2 Correlative light and electron microscopy of the compound eye of *Arca noae*. **a** Longitudinal semithin and **b** ultrathin sections through the entire compound eye. The receptor cells and pigment cells are present only in part of the epithelium. **c** Low magnification electron micrograph from the boxed field in **b** showing full length ommatidia with the receptor cells (*RC*) and adjacent pigment cells (*PC*). RCs contain a cluster of densely packed mitochondria (*DMS* distal mitochondrial segment) extending from beneath the nucleus (*Nu*) to the receptor segment (*RS*). Although in this single ultrathin section, the DMS seems to be interrupted, it represents a solitary structure as can be appreciated in the semithin section (*arrowhead* **a**; see also Fig. 3b). Below the RS, the proximal mitochondrial segment (*PMS*) can be seen. Two types of apical cell surface specializations are formed by the pigment cells (*PC*): tightly packed microvilli forming a well-organized brush border (*BB*) and loosely arranged, shorter microvilli (*MV*). The cytoplasm of distal pigment cells contains an electron dense, amorphous material along the lateral plasma membrane (*arrowheads* **c**). Additional details are presented in Fig. 5 and Supplemental Figure 1. *Scale bars* 46 μm (**a**), 24 μm (**b**), 5 μm (**c**)

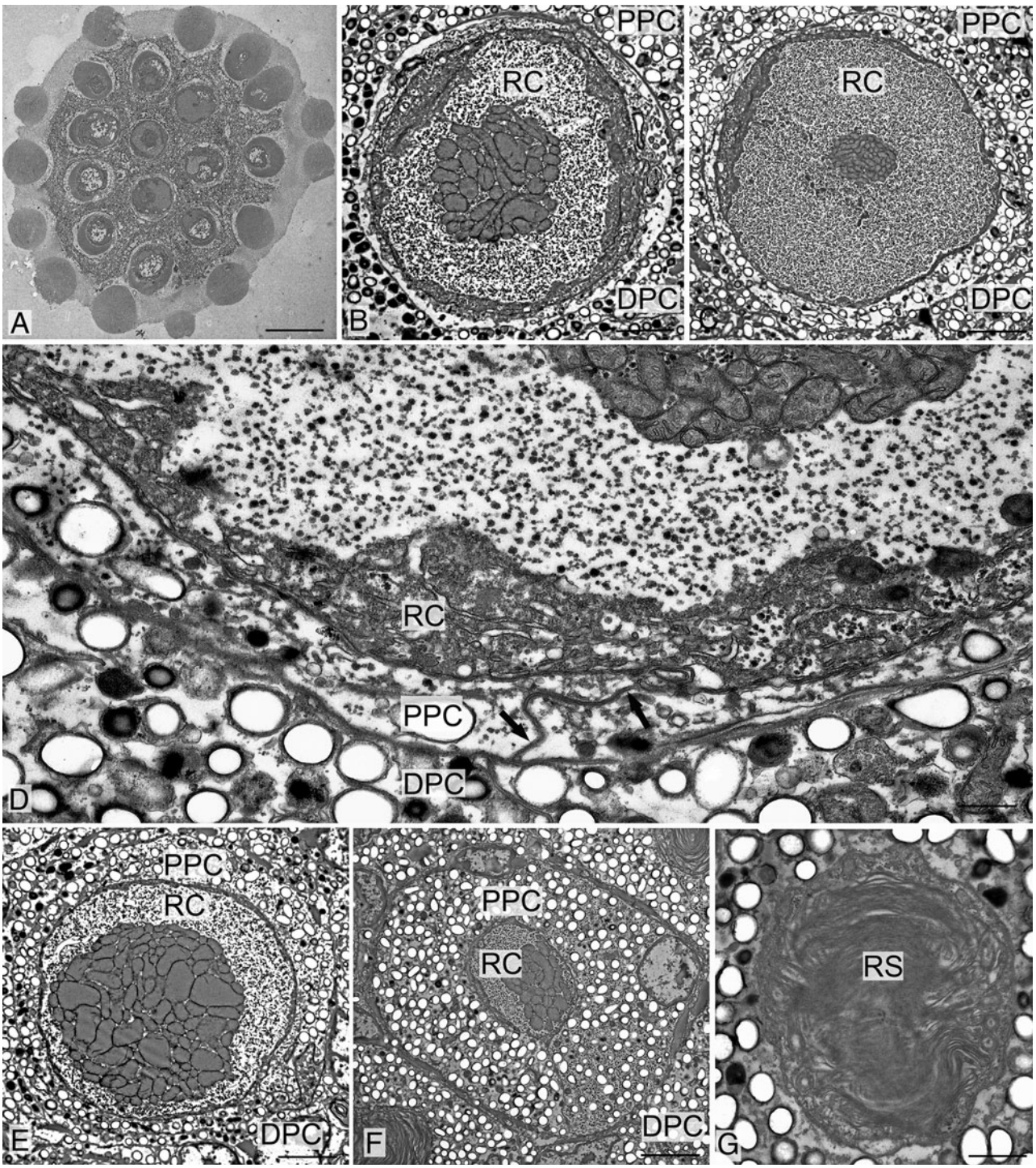

Fig. 3 **a** An oblique horizontal ultrathin section of a compound eye. **b**–**g** Cross sections of receptor cells (*RC*) with surrounding proximal (*PPC*) and distal pigment cells (*DPC*) at different infranuclear levels down to the receptive segment (*RS* **g**). **d** Higher magnification of part of a cross-sectioned RC with densely packed mitochondria, numerous electron dense granules and a small rim of cytoplasm, which is surrounded by PPC and DPC. *Arrows* point to the lateral plasma membrane of two adjacent PPC. *Scale bars* 16 μm (**a**), 2.2 μm (**b**), 2 μm (**c**), 0.5 μm (**d**), 2.4 μm (**e**), 3 μm (**f**), 1.1 μm (**g**)

Fig. 4 Cytoplasm of receptor cells. **a** The goblet-shaped infranuclear cytoplasm of a longitudinally sectioned receptor cell contains densely packed ▶ mitochondria (*DMS*) with short tubuli, and abundant highly electron dense granules. **b** A large Golgi apparatus (*GA*) with small clusters of electron dense granules in its neighborhood (*asterisk*). The cytoplasm between mitochondria of the DMS (*M*) and the Golgi apparatus is filled with such granules (*asterisk*). As shown in **c**, such granules are reactive with gold-labeled Concanavalin A in an ultrathin frozen section. For additional details, see Supplemental Figure 2. Longitudinally sectioned receptive segments (*RS*) with cross-sectioned sensory cilia (*arrowheads*) are shown in the *inset* to **a** and in **d** and **e**. *M* mitochondria, *PPC* proximal pigment cells. *Scale bars* 0.3 μm (**a**), 0.6 μm (*inset* in **a**), 0.4 μm (**b**), 0.17 μm (**c**), 0.5 μm (**d**), 0.8 μm (**e**)

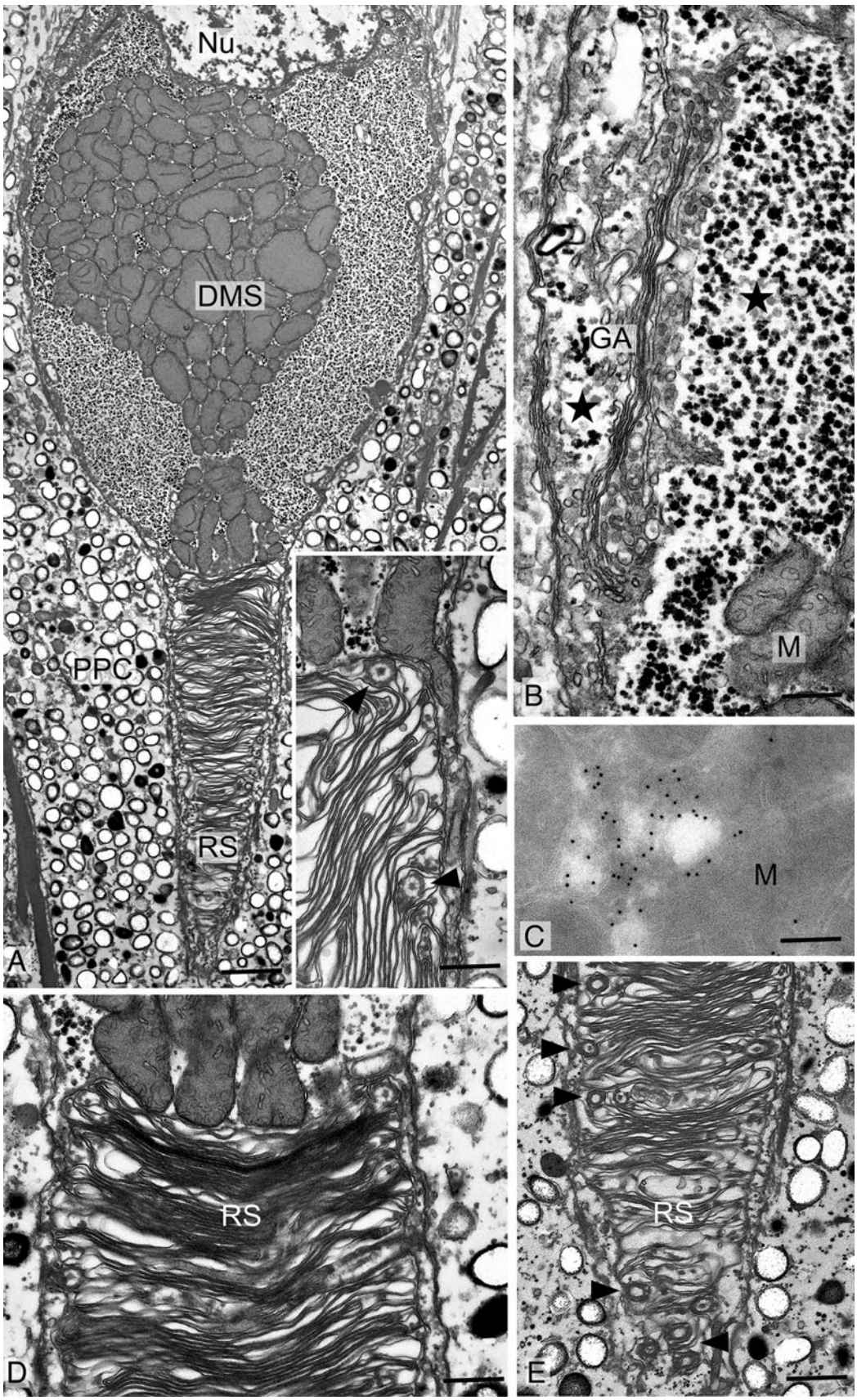

Fig. 4

Lectin–gold labeling

Tissue was fixed in 4% formaldehyde in Hepes-buffered seawater for 1 h at ambient temperature. After rinses with PBS, small tissue pieces were immersed in 2.3 M sucrose followed by freezing in liquid nitrogen. Frozen ultrathin sections were prepared at −120°C according to Tokuyasu (1978, 1986) and picked up on nickel grids, floated on droplets of PBS containing 50 mM NH_4Cl on ice for 30 min and rinsed with buffer. Lectin–gold labeling of frozen–thawed thin sections was performed according to Roth (1983) with the following modifications. Sections were incubated with Concanavalin A (2.5 µg/ml in PBS containing 1% bovine serum albumin and 0.1% Tween 20; Sigma-Aldrich) for 1 h, rinsed with buffer, incubated with antiserum against Concanavalin A (15 µg/ml in PBS containing 1% bovine serum albumin and 0.1% Tween 20; Sigma-Aldrich) for 1 h, rinsed with buffer, incubated with protein A–gold (8 nm, diluted to an $OD_{525nm} = 0.06$ with PBS containing 0.2% defatted milk and 0.01% Tween 20) for 1 h (Roth et al. 1978), rinsed with buffer and fixed with 1% glutaraldehyde in PBS for 20 min. Finally, sections were embedded in methylcellulose-uranyl acetate (Tokuyasu 1978) and observed under the electron microscope.

Results

General aspects

In this study, we have analyzed the compound eyes of *Arca noae*, which are located at the mantel edge of the animals (Fig. 1). The cup eyes were not studied. For the description of the various cell types and their substructures, we followed the nomenclature used by Nilsson (1994) in studies of other ark clams.

The convex-shaped compound eyes of *Arca noae* vary in size and sit on short stalks. In sagittal sections along the longitudinal axis of the eyes, the receptor and pigment cells form a single-layered epithelium, which can be appreciated in both semithin (Fig. 2a) and ultrathin (Fig. 2b, c) sections. Of note, the ommatidia are not present along the entire circumference of the epithelium (Fig. 2a, b). In single oblique horizontal sections through the eyes, at least 25 ommatidia can be observed (Fig. 3a). In *Arca noae*, as previously reported for *Barbatia cancellaria* and *Arca zebra* (Nilsson 1994), crystalline cones as described for arthropod and *Sabella melanostigma* compound eyes could not be observed (Fig. 2c; Suppl. Fig. 1A).

Fig. 5 The apex of receptor cells is roofed by proximal pigment cells. ▶ **a**, **b** A thin layer of cytoplasm of proximal pigment cells (*PPC*) covers the apical portion of a receptor cell (*RC*). The plasma membrane (*PM*) of RCs and PPCs are in close contact with each other and form extensive cellular interdigitations (*arrowheads* in **a**, **c**). *Arrows* in **a** and **b** point to the free apical plasma membrane of PPC. **c–f** Four consecutive serial sections from a larger series show the extensively interdigitating plasma membrane (*PM*) of two PPCs and one RC (*arrowheads* **c**). Apically, the PPCs are joined by a junctional complex (*Ju* **c**). *BB* brush border of PPCs, *NE* nuclear envelope, *Nu* nucleus of the RC. *Scale bars* 0.4 µm (**a**), 0.3 µm (**b**), 0.45 µm (**c–f**)

The receptor cells

In longitudinal sections, the receptor cells of *Arca noae* have a characteristic goblet-like shape (Fig. 2) and can be divided into four parts, like those of *Barbatia cancellaria* and *Arca zebra* (Nilsson 1994). The apical, sickle-shaped cytoplasm contains the nucleus (Figs. 2c, 6a; Suppl. Fig. 1A), cisternae of endoplasmic reticulum, a well-developed Golgi apparatus with associated small vesicles and clusters of electron dense granules, which are considered in detail below (Figs. 4b, 5a, b; Suppl. Fig. 2A, B). A narrow layer of peripheral cytoplasm reaches down to (and beyond) the receptive segment and encloses two structural elements: a cluster of mitochondria and highly electron dense granules. The numerous, densely packed mitochondria make up the distal mitochondrial segment (Figs. 2c, 3b, c, e, f, 4a). Based on the serial section analysis and as observed in fortuitous sections, the distal mitochondrial segment consists of a single, asymmetric cluster, which extends from the nucleus to the receptive segment (arrowhead in Figs. 2a, 4a, d). The mitochondria vary in size and possess few, stubby cristae (Fig. 4b, d, Suppl. Fig. 2B). The space lateral of the distal mitochondrial segment is filled with countless highly electron dense granules (Figs. 3b–f, 4a, b, Suppl. Fig. 2). Small clusters of such granules are also found between the mitochondria (Fig. 4a, Suppl. Fig. 2B). Both their size (15–40 nm in diameter) and fine structure are consistent with that of β particles of glycogen of vertebrates (Pavelka and Roth 2010). They bear no resemblance to the photic vesicles of gastropod photoreceptors (Eakin and Brandenburger 1975; Katagiri 1984). The glycogen nature of these particles was also supported by their reactivity with Concanavalin A (Fig. 4c), a plant lectin with high affinity for glycogen (Goldstein and Poretz 1986; Roth 1983). As observed in other species of ark clams, the receptive segment consists of staples of flat membrane discs (Fig. 2c, 4a, d, e) derived from sensory cilia (Fig. 4a, d, e). It is followed by the proximal mitochondrial segment composed of a few, loosely arranged mitochondria (Fig. 2c) and by the axon. Accessory receptor cells, as reported for *Barbatia cancellaria* (Nilsson 1994), were not observed.

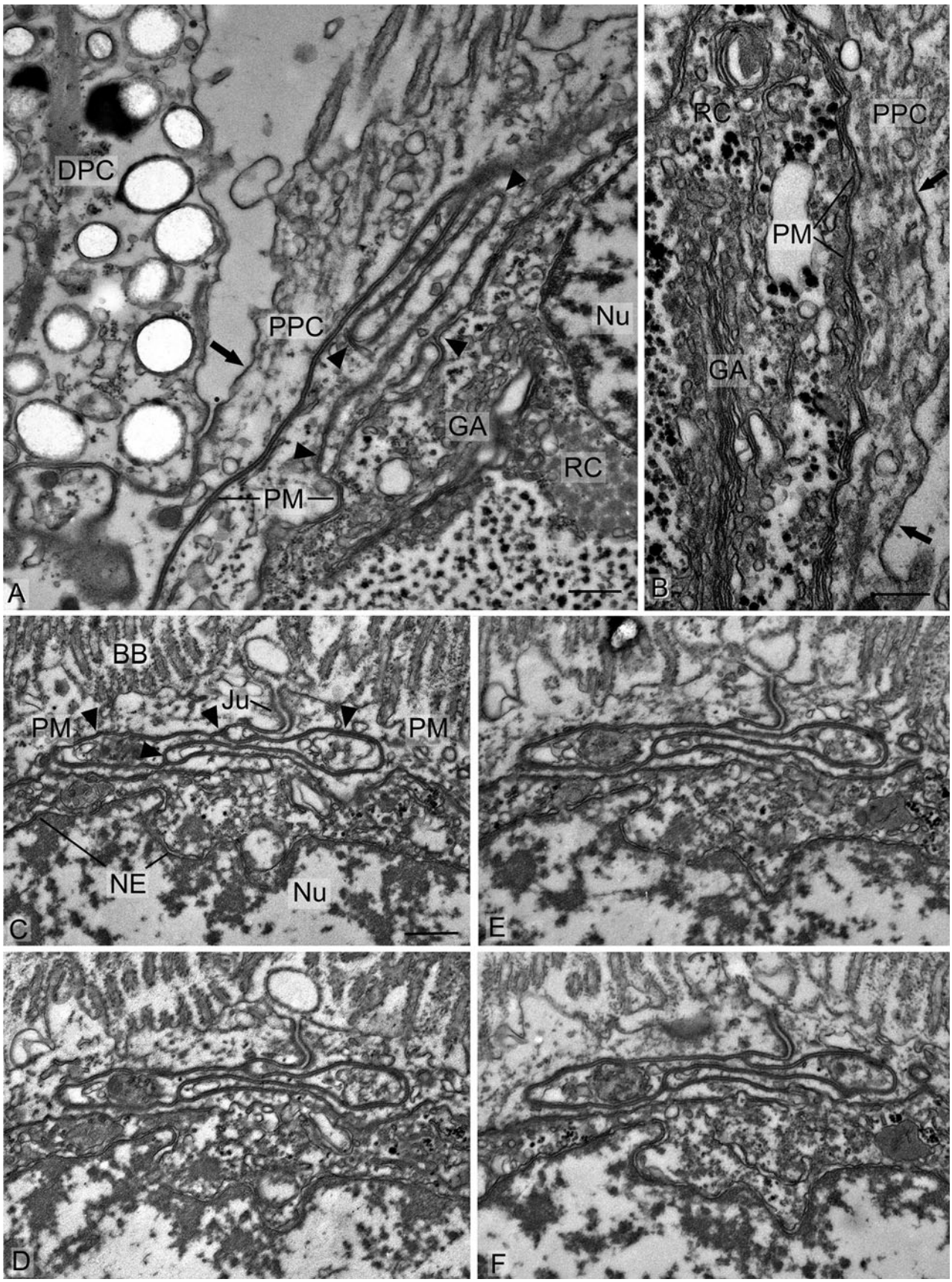

Fig. 5

Receptor cells are surrounded and covered by proximal pigment cells

Series of consecutive longitudinal or horizontal ultrathin sections revealed that at least two proximal pigment cells cover the apex of the receptors cells and wrap around them down to their base (Figs. 2c, 3, Suppl. Fig. 1A). Contacts between receptor cells and distal pigment cells could not be observed. The non-pigmented upper part of proximal pigment cells and the receptor cell were interlinked by narrow plasma membrane folds (Fig. 5). The complexity and the intensity of their plasma membrane interlocking could be best appreciated in serial sections (Fig. 5c–f). In addition, the proximal pigment cells were linked by junctional complexes (Figs. 5c–f, 6c).

A unique feature of proximal pigment cells: microlamellar bodies

The apical plasma membrane of proximal pigment cells formed an impressive brush border composed of ≈9–11 μm long aligned microvilli (Figs. 2, 6, Suppl. Fig. 1). In contrast, adjacent distal pigment cells exhibited shorter and fewer, irregularly arranged microvilli (Figs. 2, 6, Suppl. Fig. 1). The microvilli of proximal pigment cells have a diameter of ≈70–80 nm and a central core of filaments, most probably actin filaments (Fig. 6b, g). A closer inspection at high primary magnification (≥30,000×) revealed spectacular structures at the extracellular surface of the microvilli, namely a huge number of what we call microlamellar bodies (Fig. 6e–g, Suppl. Fig. 3, 4). They are composed of alternating electron dense (up to 5) and lucent bands. These ultrastructural features were observed both in ultrathin sections of aldehyde-osmium tetroxide-fixed and Epon-Araldite-embedded eyes as well as in ultrathin frozen sections from only aldehyde-fixed eyes. In Epon-Araldite ultrathin sections, the microlamellar bodies had a size of ≈25–30 nm. They were present along the microvilli with the exception of their uppermost part and the tips (Fig. 6e, Suppl. Fig. 4). Clusters of large vesicles in the apical cytoplasm of proximal pigment cells were their probable source as they contained a vast number of microlamellar bodies (Fig. 6a, c, d, Suppl. Fig. 5). The large, presumptive secretory vesicles were not positive for acid phosphatase (not shown). The structure of the microlamellar bodies was comparable in the presumptive secretory vesicles and in the brush border of proximal pigment cells. Although measurement of microlamellar bodies proved difficult in the presumptive secretory vesicles, their size was ≈40–50 nm and up to nine electron dense bands were counted. Exocytosis of the vesicle content could not be observed, but their close contact with the plasma membrane suggests that this may occur, though infrequently (Fig. 6d). Incubation of ultrathin frozen sections with Concanavalin A, which is reactive with terminal and internal glucose and mannose residues of glycoproteins and glycolipids (Goldstein and Poretz 1986; Williams et al. 1981) produced no labeling of the microlamellar bodies notwithstanding intense labeling of the remainder intermicrovillar space (Fig. 6h, i).

Fig. 6 Apical plasma membrane differentiation of pigment cells. **a**, **b** Proximal pigment cells (*PPC*) covering a receptor cell (*RC*) form a highly ordered brush border (*BB*), whereas the adjacent distal pigment cells (*DPC*) exhibit numerous loosely arranged microvilli (*MV*). The microvilli differ in diameter as can be seen in a cross section (**b**). For additional details, see Supplemental Figure 1. The PPC contains large vesicles (*arrowheads* **a**). **c**, **d** Such vesicles (*MLB* **c**, **d**) contain numerous, densely packed microlamellar bodies composed of parallel arranged electron dense and lucent bands (*insets* in **c** and **d**). Occasionally, the vesicle limiting membrane is in close contact with the apical plasma membrane (*arrowhead* **d**). For additional details, see Supplemental Figure 5. In longitudinal and cross sections (**e**–**g**), the brush border-forming microvilli are covered by microlamellar bodies. In contrast, microlamellar bodies are not observed along the microvilli of DPC (**b**). For additional details, see Supplemental Figures 3 and 4. **h**, **i** In ultrathin frozen sections, microlamellar bodies of the brush border (*arrowheads* **h**) are not reactive with gold-labeled Concanavalin A. *Arrow* in **c** points to a junctional complex of two PPCs. *Scale bars* 1.6 μm (**a**), 0.15 μm (**b**), 0.16 μm (**c**), 0.1 μm (**d**), 0.12 μm (**e**), 0.1 μm (**f**), 0.08 μm (**g**), 0.14 μm (**h**), 0.08 μm (**i**)

When living compound eyes on dissected mantel edges were observed in seawater from vertically above, the eyes had a sponge-like appearance because the ommatidia exposed to vertical incident light did not absorb visible light (Fig. 1). This indicates that structures of the ommatidia transmitted significantly more light than the surrounding pigment cells. Interestingly, the ommatidia in the margins of the eyes that were exposed to oblique light refracted the incident light (Fig. 1).

Distal pigment cells contain plasma membrane-associated sheets

The two types of pigment cells differ not only by their microvilli and their arrangement but also in their spatial relation to the receptor cells. Distal pigment cells were arranged around the proximal ones, which on their part wrapped around the receptor cells (Figs. 2, 3, 5, 7, Suppl. Fig. 1). Hence, proximal and distal pigment cells are arranged in two distinct layers around the receptor cells. The entire cytoplasm of distal pigment cells was filled with pigment granules in contrast to proximal pigment cells, and a dense, amorphous material, which was often attached to the lateral plasma membrane, was regularly observed (Figs. 2c, 7). By analyzing the shape of the amorphous material in both longitudinal and horizontal thin sections, it became obvious that it consisted of sheets. In fortuitous longitudinal sections, such sheets could be followed along

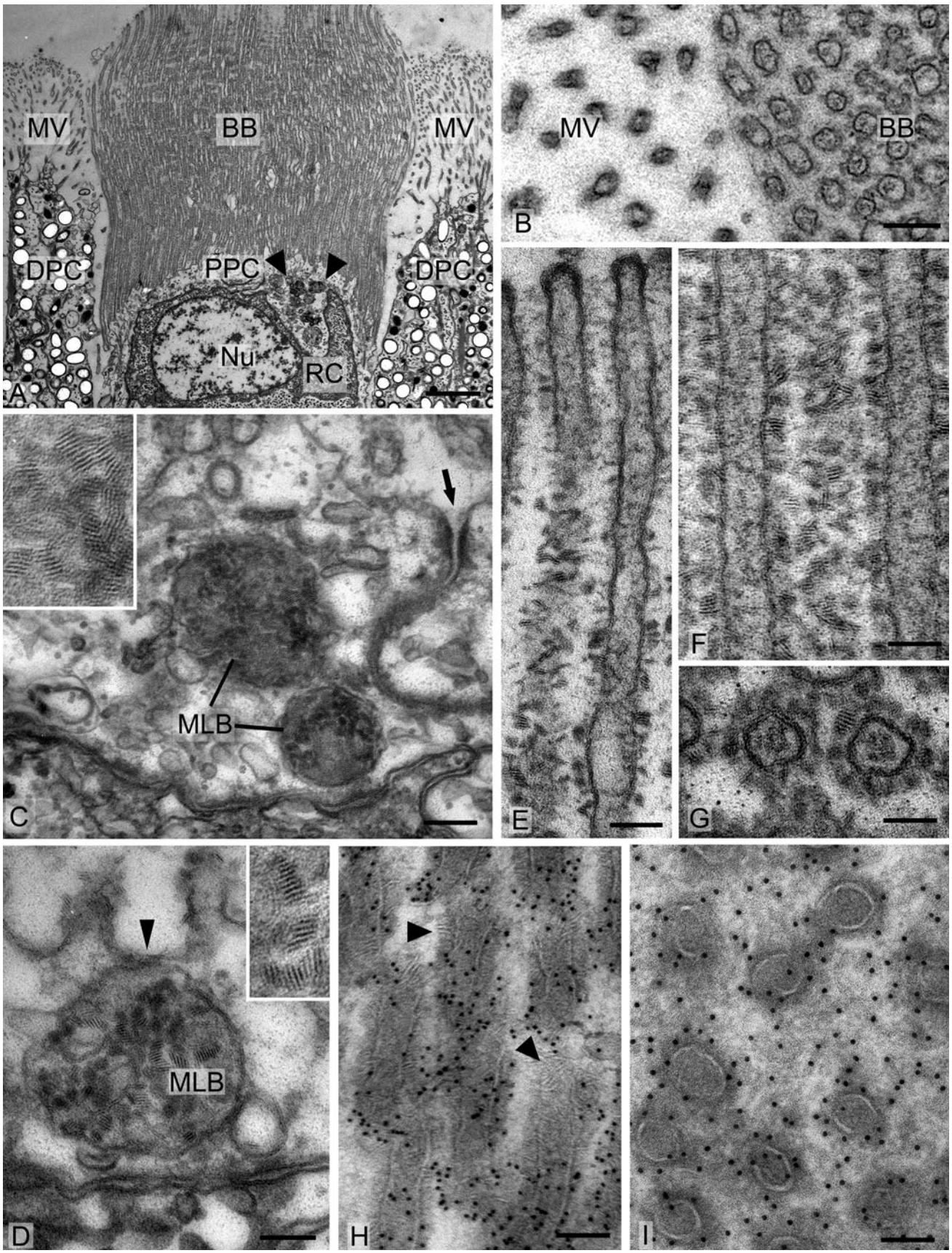

Fig. 6

the entire lateral plasma membrane of distal pigment cells (Fig. 7c).

Discussion

The present electron microscopic and serial section analysis establishes the ommatida of *Arca noae* compound eyes as a three-tiered structure (Fig. 8). Single receptor cells correspond to tier I. They are wrapped and their apex is covered by proximal pigment cells representing tier II. The distal pigment cells form tier III. Our study has revealed hitherto unknown features of the proximal pigment cells and the receptor cells. The proximal pigment cells, which fully cover the receptor cells with a thin layer of cytoplasm, have a well-aligned brush border with its microvilli covered by countless microlamellar bodies. The infranuclear cytoplasm of the ciliary receptor cells contains a mass of glycogen particles that surround the distal mitochondrial segment.

Since the initial fixation conditions are important for optimal fine structural preservation (Hayat 2000; Maunsbach and Afzelius 1999), a few technical remarks are appropriate in view of our new findings. The compound eyes of *Arca noae* and of other ark clams have a single-layered epithelium, which is a very favorable situation for chemical fixation by immersion. Since the osmolarity of the fixative vehicle is important, seawater was used in the previous (Nilsson 1994) and present studies. We fixed the eyes in a mixture of formaldehyde and glutaraldehyde, and not in glutaraldehyde alone, and at ambient temperature. The reason for this was twofold. First, aldehydes penetrate faster at ambient temperature than in the cold. Second, formaldehyde penetrates cells and tissues faster than glutaraldehyde and does not generate an osmotic gradient. A mixed formaldehyde–glutaraldehyde fixative therefore combines rapid initial fixation and metabolic arrest by the monoaldehyde with slower penetration and more efficient protein cross-linking by the dialdehyde. This together resulted in an excellent fine structural preservation of our specimens, particularly of the pigment cell's brush borders. It is also important to note that the microlamellar bodies of the brush border and in the presumptive secretory vesicles of proximal pigment cells were observed, and with an identical fine structure, both in ultrathin sections after resin embedding and in ultrathin frozen sections after sucrose infiltration. However, to be recognized and resolved, these minute structures require observation at high ($\geq$30,000×) primary magnification in the electron microscope and it would be worth a try to verify their existence in other ark clams.

We are confident that microlamellar bodies are not artifacts for reasons briefly outlined above and because of their presence in presumptive secretory granules in proximal pigment cells. Although there composition remains to be determined, their lamellar structure resembles that of liquid crystalline phospholipids. In vertebrates, at least two instances are known of secretory granules whose phospholipid content fulfills vital functions after secretion. The type II alveolar epithelial cells of the lung synthesize and store the pulmonary surfactant in specific secretory granules (Dietl and Haller 2005; Orgeig et al. 2004; Pavelka and Roth 2010; Vanhecke et al. 2010). Their main phospholipid content is the surface-active dipalmitoylphosphatidylcholine. Following secretion, a surface tension-reducing phospholipid monolayer forms at the air–liquid interface, which prevents the collapse of the alveolar airspace. The other example relates to the skin. In the skin of terrestrial creatures, secreted phospholipids constitute a major component of the epidermal permeability barrier, which prevents excessive water loss and hence life-threatening desiccation (Elias and Menon 1991; Madison 2003; Oashi et al. 1973). The keratinocytes of the granular layer have secretory granules, the Odland bodies, that are filled with short stacks of lipid lamellae (Pavelka and Roth 2010). After secretion, the lipids are processed by co-secreted lipid hydrolases to yield a mixture of ceramide, cholesterol and free fatty acids. This processing results in the formation of large lamellar lipid sheets in the intercellular space, which become covalently linked to the intracellular cornified protein envelope of corneocytes. These two examples illustrate not only the existence of secretory granules with lipids as their main content but also that secreted phospholipids can be important for the normal structure and function of tissues like lung and skin.

The microlamellar bodies of proximal pigment cells retain their characteristic fine structure after release in the extracellular space, in contrast to the lipids secreted by alveolar type II epithelia and keratinocytes. The composition of the microlamellar bodies is unknown as are their possible optical properties. Because of their characteristic appearance, it is tempting to speculate that phospholipids may be a major component. Currently, one can only speculate about the function(s) they may fulfill. Both their intimate contact with the microvillar membrane and their dense packing could indicate a function as a scaffold to

Fig. 7 Relationship between pigment cells and receptor cells. ▶ **a–d** Both, proximal pigment cells (*PPC*) and distal pigment cells (*DPC*) extend below the receptor segment (*RS*) of receptor cells (*RC*). In DPC, in contrast to PPC, sheets of a homogenous electron dense material are present (*arrowheads* **a–f**), which are often attached to the plasma membrane. **c** In a fortuitous longitudinal section, such sheets (*arrowheads*) can be followed from the base to the apex of DPC. In addition, such material can be observed in a perinuclear locale (*arrowhead* **e**) and in the cytoplasm (*arrowhead* **f**). *Nu* nucleus. *Scale bars* 2 μm (**a**), 2.2 μm (**b**, **c**), 1.35 μm (**d**), 0.5 μm (**e**), 0.53 μm (**f**)

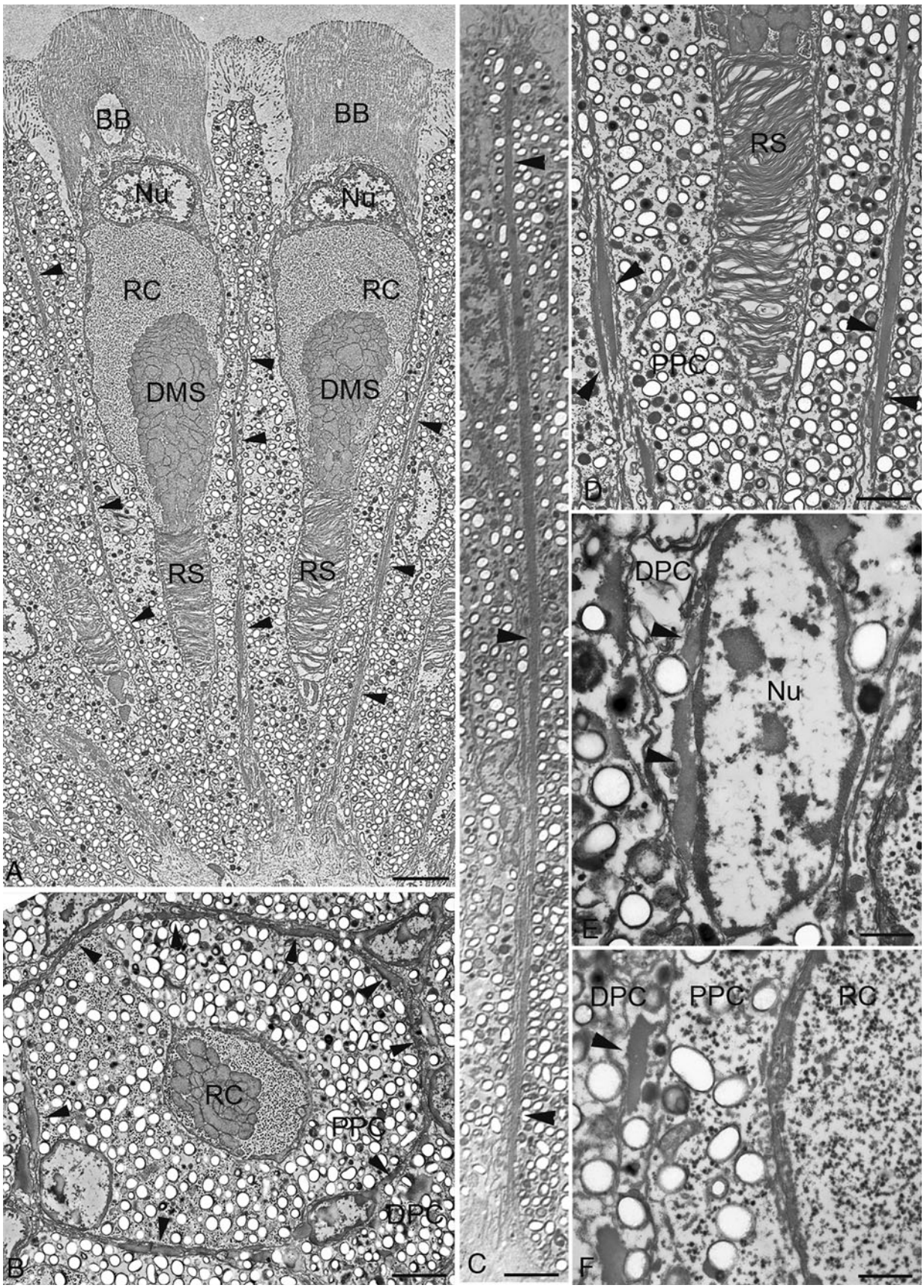
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Fig. 7

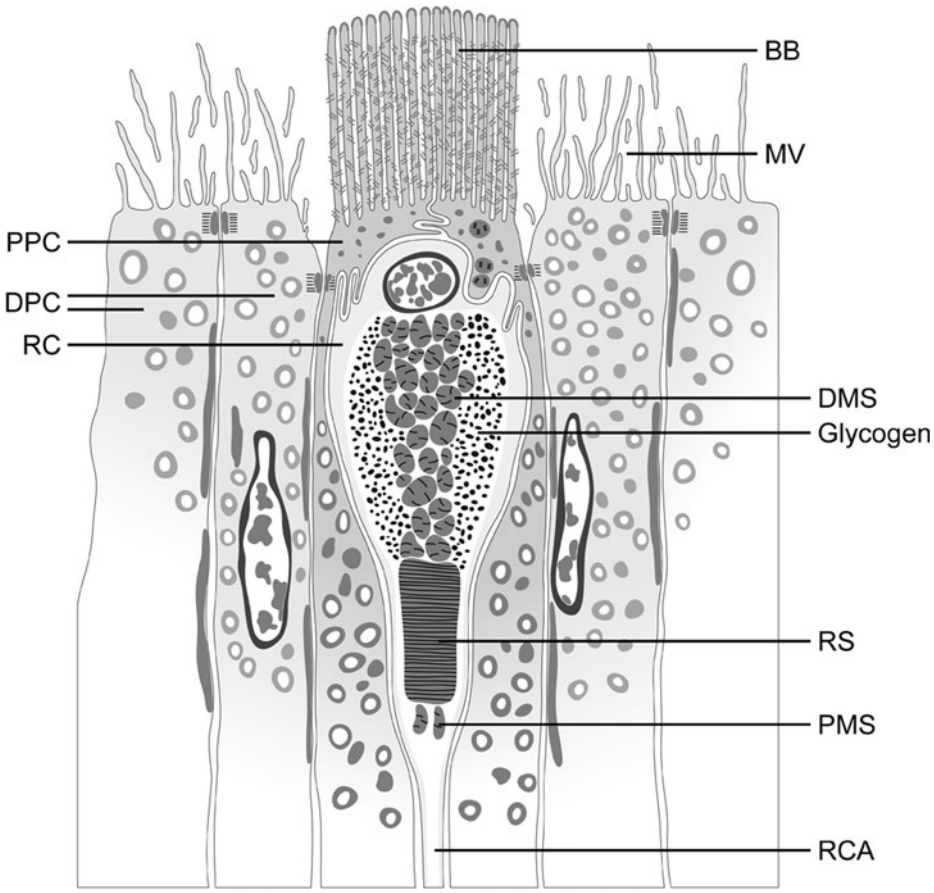

Fig. 8 Schematic illustration of an ommatidium of *Arca noae*. *BB* brush border, *DMS* distal mitochondrial segment, *DPC* distal pigment cell, *MV* microvilli, *PMS* proximal mitochondrial segment, *PPC* proximal pigment cell, *RC* receptor cell, *RCA* receptor cell axon, *RS* receptor segment

maintain the microvilli of the brush border properly aligned. This brings up the question why, as compared to the distal pigment cells, the proximal pigment cells have such a highly organized brush border. We would like to propose that it is of significance for guiding incident light to the underlying receptor cells. Noteworthy, the receptor cells are covered by a very thin layer of proximal pigment cell cytoplasm, which is free of pigment and organelles, except occasional small clusters of secretory granules with microlamellar bodies. In this context, the recent characterization of Müller cells in the mammalian retina as living optical fibers that provide a low-scattering passage for light to the photoreceptor cells offers an intriguing possibility (Franze et al. 2007). Similarly as observed and measured by Franze et al. (2007) for Müller cells, the brush border of proximal pigment cells transmits significant more light than the surrounding distal pigment cells, which relates to the sponge-like appearance of the compound eyes of *Arca noae*. The fairly regular geometry of the brush border microvilli, in remote analogy to the system of parallel arranged fiber-like shaped Müller cells, and its perfect alignment over a single receptor cell occurs to provide a light-guiding structure. Hence, the brush border can be considered as a simple optical fiber rather than an extracellular lens as proposed for the crystalline cone of sabellid polychaetes (Kerneis 1973; Nilsson 1994). Measurement on dispersed compound eyes of the ark *Barbatia cancellaria* showed that the microvillar body had actually "a lower refractive index than most other cellular components" (Nilsson 1994), which fully supports our notion.

An prominent feature of the receptor cells is the distal mitochondrial segment, which extends as a compact mass from the nucleus to the receptive segment and the mass of glycogen surrounding it. Mitochondria are known to be highly scattering structures, and glycogen is not optical inert either. However, which optical properties the combination of the two may have is unknown, although it appears unlikely that they absorb light to a great extent. Apparently, pigment cells of the compound eyes are not transparent due to the presence of pigment. The sheets of unidentified amorphous material specific for distal pigment cells along their lateral plasma membrane are a reasonable candidate as a longitudinal stiffener for the slender pigment cells rather than a chromophore or a light reflecting structure.

The photoreceptors of compound eyes of sabellid polychaetes and ark clams including *Arca noae* are of the ciliary type and provide an optical alarm system. For it to

be highly efficient, we propose the brush border of the proximal pigment cells of *Arca noae* to serve as a low-scattering optical fiber for the receptor cells.

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